Citric acid and salts

Handling/Processing

Identification of Petitioned Substance

Chemical Names:
Citric acid, calcium citrate, potassium citrate, sodium citrate

Other Names:
Citric acid: 2-hydroxypropane-1,2,3-tricarboxylic acid, 3-carboxy-3-hydroxypentanedioic acid
Calcium citrate: 2-hydroxy-1,2,3-propanetricarboxylic acid, 2-hydroxy-1,2,3-propane- tricarboxylic acid calcium salt (2:3)
Potassium citrate: tripotassium citrate, potassium citrate tribasic, potassium citrate tribasic monohydrate
Sodium citrate: sodium dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate, disodium hydrogen 2-hydroxypropane-1,2,3-tricarboxylate, trisodium citrate, and trisodium 2-hydroxypropane-1,2,3-tricarboxylate

Trade Names:
There are no trade names for the pure chemicals.

CAS Numbers:
77-92-9 (citric acid), 813-94-5 (calcium citrate) (also is listed as 813-994-95 in 21 CFR Sec 184.1195), 5785-44-4 (calcium citrate tetrahydrate), 866-84-2 (potassium citrate), 6100-05-6 (potassium citrate tribasic monohydrate) (also is listed as 6100-905-96 in 21 CFR §184.1625), 18996-35-5 (monosodium citrate), 144-33-2 (disodium citrate), 68-04-2 (trisodium citrate) (also is listed as 68-0904-092 in 21 CFR §184.1751), 6132-04-3 (trisodium citrate dihydrate), 6858-44-2 (trisodium citrate pentahydrate)

Other Codes:
E330 (citric acid), E333 (calcium citrate), E332 (potassium citrate), E331 (sodium citrate)

Summary of Petitioned Use

Citric acid is listed at §205.605(a) as a nonagricultural (nonorganic) allowed nonsynthetic under 'acids', with the annotation that it must be produced by microbial fermentation of carbohydrate sources. Citric acid is also permitted for the acidification of sodium chlorite, as listed at §205.605(b). The citrate salts (calcium, potassium, and sodium) are also listed at §205.605(b) as nonagricultural (nonorganic) allowed synthetics. Citric acid is additionally listed at §205.601 as a pH adjuster for liquid fish products under synthetic substances allowed for use in organic crop production. For the purposes of this review, the free acid and the various salts will be grouped together and referred to as citric acid, except when it is appropriate to break them out as separate compounds.

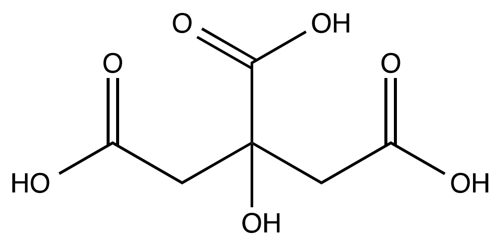
Citric acid is used as a food ingredient in the production of fruit products, juices, oils and fats, and for many other food products where it functions as an acidulant, pH control, flavoring and sequestrant. It is also used as a dispersant in flavor or color additive products. In addition, it is used to wash processing equipment to eliminate off-flavors.

Calcium citrate is used as an ingredient in dietary supplements, and as a nutrient, sequestrant, buffer, antioxidant, firming agent, acidity regulator (in jams and jellies, soft drinks and wines), as a raising agent and an emulsifying salt. It is also used to improve the baking properties of flours and as a stabilizer. Potassium and sodium citrate are used as ingredients where they function as acidulants, pH controls, flavoring agents, sequestrants, and buffering or emulsifying agents. Potassium citrate is used to replace sodium citrate whenever a low sodium content is desired. These materials are also used as dispersants in flavor or color additives. In addition they are used to wash processing equipment in order to eliminate off-flavors.

Characterization of Petitioned Substance

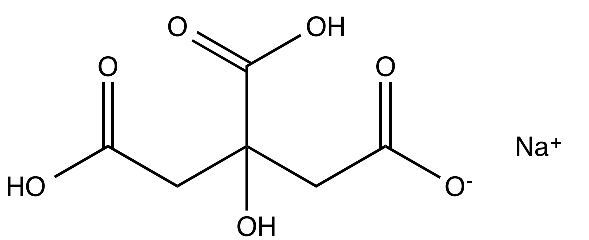
Composition of the Substance:

Citric acid is a naturally occurring non-volatile organic acid with the molecular formula $C_6H_8O_7$ and the following structure:

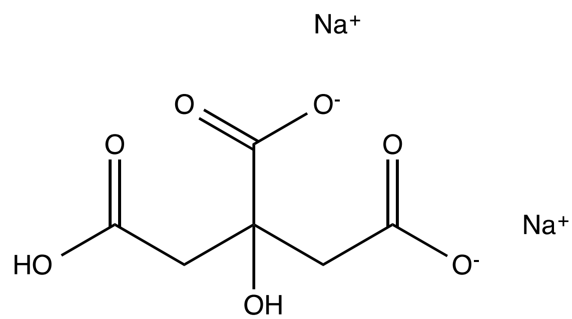


citric acid

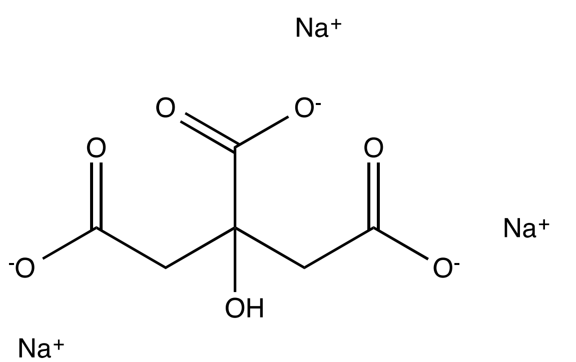
Figure 1: Citric acid molecular structure (ChemBioDraw 2014)



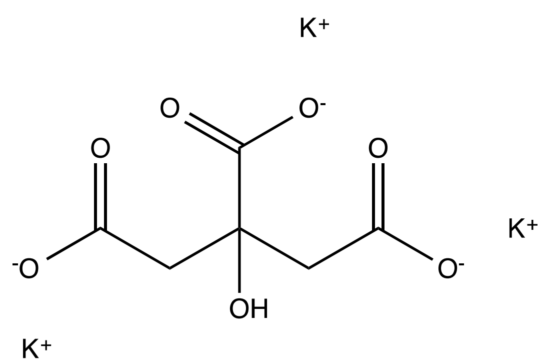
mono sodium citrate



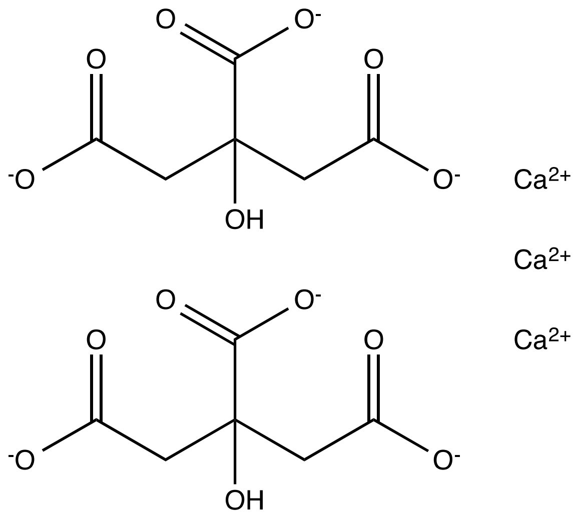
disodium citrate



tri-sodium citrate



potassium citrate



calcium citrate

Figure 2: Citrate salts molecular structures. (ChemBioDraw 2014)

The citrate salts come with various levels (mono-, di-, tri-) of the metal cations (calcium, potassium or sodium) and various states of hydration. Examples of representative structures are shown above (Figure 2).

<u>Source or Origin of the Substance:</u>
Citric acid is a naturally produced non-volatile organic acid. For the purposes of this review, production by microbial fermentation with *Aspergillus niger* or *Candida* yeasts from carbohydrate sources will be the focus, although some additional information regarding production from plant sources is included. The citrate salts are all produced by chemical reaction with citric acid and the hydroxide or carbonate of the respective salt (calcium, sodium or potassium).

<u>Properties of the Substance:</u>
Citric acid is a clear to white crystalline solid. It is odorless and has a strong acidic (sour) taste. The citrate salts are clear to white crystalline solids with an acidic (sour) taste, with some having a slightly salty taste.

Table 1. Chemical properties of citric acid and citrate salts (Furia 1973; U.S. National Library of Medicine 2014; Weast 1985)

Citric Acid		
Chemical Formula	$C_6H_8O_7$	
Molecular Weight	192.124 g/mole	
Physical Aspects	from cold water	colorless, translucent ortho-rhombic
	from hot water	anhydrous, colorless, translucent holohedral class of monoclinic crystals
	Monohydrate	rhombic crystals
Melting Point	Anhydrous	153°C
	Monohydrate	softens at 70-75°C when heated slowly and melts completely at 135-152°C
		With rapid heating the monohydrate liquefies at 100°C
Boiling Point	Decomposes	
Solubility	water	54.0% w/w at 10°C
		59.2% at 20°C

		64.3% at 30°C
		68.6% at 40°C
		70.9% at 50°C
		73.5% at 60°C
		76.2% at 70°C
		78.8% at 80°C
		81.4% at 90°C
		84.0% at 100°C
	Ethanol, 25°C	58.9 g/100ml
	Ether, 25°C	1.84 g/100ml
Density	Monohydrate	1.542 g/cm^3
	Anhydrous	1.665 g/cm^3
Molecular Refraction	Monohydrate	67.11
Refractive Indexes, n_D^{20}		1.493, 1.498, 1.509 (hydrate)
Heat of Combustion, $\Delta H°_c$	Monohydrate	-471.4 kcal/mole
	Anhydrous	-474.5 kcal/mole
Bulk Density	Anhydrous	56.2 lbs./cu ft.
Ionization Constants	K1	8.2 x 10^{-4}
	K2	1.8 x 10^{-5}
	K3	3.9 x 10^{-6}
	pK_a1	3.13
	pK_a2	4.76
	pK_a3	6.39
Heat of Solution, 25°C		-3.9 kcal/mole
Viscosity, (50% Aqueous solution, 25°C)		6.5 cP
Std. Free Energy of Anion Formation ($\Delta F°_f$), 25°C		-278.8 kcal for aqueous solutions
Buffering Index		2.46
Odor		Odorless
Taste		Tart, strongly acidic taste, pleasant sweet tart
LD_{50}	Rats, oral	3000-12,000 mg/kg
Calcium Citrate		
Chemical Formula	$Ca_3(C_6H_5O_7)_2$	
Molecular Weight	Anhydrous	498.4334 g/mole
	Tetrahydrate	570.49452 g/mole
Physical Aspects	Appearance	white needles or powder
Melting Point		120°C
Boiling Point	Decomposes	
Solubility	water	0.085 g/100ml at 18°C, 0.096 g/100ml at 23°C
	Ethanol	0.0065 g/100ml
Potassium Citrate		

Chemical Formula	Tribasic	$K_3C_6H_5O_7$
	Tribasic monohydrate	$K_3C_6H_5O_7 \cdot H_2O$
	Monobasic	$KH_2C_6H_5O_7$
Molecular Weight	Tribasic monohydrate	324.41 g/mole
	Monobasic	230.22 g/mole
Physical Aspects	Appearance	white powder, hygroscopic
Melting Point		180°C
Boiling Point		230°C
Solubility	Monohydrate-water	167 g/100ml
	Ethanol	slightly soluble
	Monobasic-water	soluble
Density	Monohydrate	1.98 g/cm³
Ionization Constants	pK_a	8.5
LD_{50}	IV, dog	170 mg/kg
Sodium citrate		
Chemical Formula	Monosodium	$NaC_6H_7O_7$
Molecular Weight	Monosodium	214.11 g/mole
Chemical Formula	Disodium	$Na_2C_6H_6O_7$ or $Na_2HC_3H_5O(COO)_3)$
Molecular Weight	Disodium	236.09 g/mole
Chemical Formula	Trisodium	$Na_3C_6H_5O_7$
	Trisodium dihydrate	$Na_3C_6H_5O_7 \cdot 2H_2O$
	Trisodium pentahydrate	$Na_3C_6H_5O_7 \cdot 5H_2O$
Molecular Weight	Trisodium anhydrous	258.06 g/mole
	Trisodium dihydrate	294.10 g/mole
	Trisodium pentahydrate	348.15 g/mole
Physical Aspects	Trisodium	white powder
Melting Point	Trisodium	>300°C hydrates lose water ca. 150°C
Solubility	Trisodium dihydrate-water	72 g/100ml at 25°C, 167 g/100ml at 100°C
	Trisodium dihydrate-alcohol	0.625 g/100ml
	Trisodium pentahydrate-water	92.6 g/100ml at 25°C
Density	Trisodium	1.7 g/cm³
	Trisodium pentahydrate	1.857 g/cm³

Specific Uses of the Substance:

Citric acid is very widely used in food processing. It is used as an ingredient, acidulant, pH control agent, flavoring, and as a sequestrant. It is used as a dispersant in flavor or color additives. It is an ingredient in dietary supplements and a nutrient, sequestrant, buffer, antioxidant, firming agent, acidity regulator (in jams and jellies, soft drinks and wines), raising agent and emulsifying salt for many other products. It is also used to improve baking properties of flours, and as a stabilizer.

Sodium citrate is used as an emulsifier in dairy products to keep fats from separating, and in cheese making where it allows the cheeses to melt without becoming greasy.

Calcium citrate provides calcium in nutritive supplements, and it can also be used as a water softener due to its chelation properties. It is used to wash processing equipment in order to eliminate off flavors, and as a pH adjuster and chelator in cleaning and sanitizing products. It is also used for its chelating properties to remove scale from boilers, evaporators and other processing equipment. Calcium citrate is widely used in cosmetic and personal care products for many of these same functions.

Potassium citrate is used as an antioxidant, acidulant, pH control, flavoring, sequestrant, emulsifying salt, stabilizer, and as a dispersant in flavor or color additives. It is also used to wash processing equipment to remove off flavors.

Approved Legal Uses of the Substance:

Citric acid is listed under 21 CFR Part 184.1033 as Generally Recognized as Safe (GRAS). The listing allows its production from lemon or pineapple juice; through microbial fermentation from *Candida spp.*; or by solvent extraction from *Aspergillus niger* fermentation. It is allowed for use in food with no limitations other than good manufacturing practice. Additionally, sections 21 CFR 173.160 and 173.165 list *Candida guilliermondii* and *Candida lipolytica* as allowed organisms for production of citric acid through microbial fermentation. The regulation requires that the citric acid produced conforms to the specifications of the Food Chemicals Codex (Food Chemicals Codex, 2010).

Section 21 CFR 173.280 covers the solvent extraction purification of citric acid from *Aspergillus niger* fermentation. This process is discussed in detail under Evaluation Question #1 in the section on recovery of citric acid. Current good manufacturing practice (GMP) for solvents results in residues not exceeding 16 parts per million (ppm) n-octyl alcohol and 0.47 ppm synthetic isoparaffinic petroleum hydrocarbons in citric acid. Tridodecyl amine may be present as a residue in citric acid at a level not to exceed 100 parts per billion.

The EPA listed citric acid and its salts in the 2004 List 4A (minimal risk inerts). The EPA allows citric acid as an active ingredient in pesticide products registered for residential and commercial uses as disinfectants, sanitizers and fungicides (EPA R.E.D. 1992) and it is exempt from tolerances per 40 CFR 180.950. Products containing citric acid in combination with other active ingredients are used to kill odor-causing bacteria, mildew, pathogenic fungi, certain bacteria and some viruses, and to remove dirt, soap scum, rust, lime and calcium deposits. Citric acid products are used in facilities, and in or on dairy and food processing equipment.

Action of the Substance:

Citric acid is very widely used in food products. It has a number of functions, including pH control and adjustment, chelation, emulsification, and as a firming agent. It functions as a pH control and buffer because of its three carboxylic acid groups, with three well-spaced pKa's (acid dissociation constant at logarithmic scale) of 3.13, 4.76, and 6.39. This allows it to buffer the pH over a wide range of pH values.

Its chelation function is again due to the multiple carboxylic acid groups that bind to metals. It typically acts in conjunction with calcium ions as a firming agent, where it binds to the calcium ions that in turn bind to pectins, proteins or other polymers, forming an ionic cross-linked structure that provides product firmness (New EcoCyc, 2014).

Combinations of the Substance:

Citric acid and its salts are most widely used on their own, but may be a major component of flavor or color products where they act as dispersants. Citric acid and its salts are commercially supplied as pure compounds and otherwise do not contain ancillary substances (Kristiansen, et al. 1999).

Status

Historic Use:

Citric acid was one of the first organic acids identified and isolated. It was first isolated from lemon juice in 1784 by Carl Scheele, a Swedish chemist. Lemon and other citrus juice had been used historically for acidification and flavoring. With the purification of citric acid as the principal agent of these properties came widespread use in food products, initially for its flavor characteristics and as an acidulant and pH control, and later for other properties such as chelation and sequestration. Citric acid was commercially produced from Italian lemons from about 1826 until 1919, when production shifted to fermentation using *Aspergillus niger*. Today, roughly 75% of citric acid production is used by the food industry, with 10% used by the pharmaceutical and cosmetic industry and the remaining 15% for industrial purposes (Kristiansen, et al. 1999).

Citric acid has been one of the principle acidulants used in food products from the inception of food processing. It was included as an allowed nonagricultural ingredient in the original organic regulations published in 2000. It was reviewed by a technical advisory panel (TAP) in 1995 as part of the review by the National Organic Standards Board for the National List.

Organic Foods Production Act, USDA Final Rule:

Citric acid is not specifically listed in OFPA. Citric acid (but not the salts) was TAP reviewed in 1995 as part of the process leading to its inclusion in the initial National List. Citric acid (produced by microbial fermentation of carbohydrate substances) is listed as an allowed nonagricultural, nonsynthetic substance at §205.605 (a), and the citrate salts are listed as nonagricultural, synthetic substances at §205.605 (b).

International

Citric acid is listed as an allowed ingredient in all international standards reviewed. Some have annotations or limitations on its use, but these are in line with the expected uses of citric acid. The citrate salts are generally listed as allowed, but with restrictions associated with their usage. Details are noted below under the various standards.

Canada - Canadian General Standards Board Permitted Substances List

http://www.tpsgc-pwgsc.gc.ca/ongc-cgsb/programme-program/normes-standards/internet/bio-org/documents/032-0311-2008-eng.pdf

Citric acid is allowed per Table 6.3 of the Canada Organic Regime (COR) Permitted Substances List (CAN/CGSB 32.311). It is listed under "Acids: citric–produced by microbial fermentation of carbohydrate substances." Later in the same section, citric acid is allowed "from fruit or vegetable products." The Permitted Substances List also specifies 'organic citric acid' in the list of acidulants for liquid fish products as soil amendments or for crop nutrition (Table 4.2).

Iron citrate is allowed as an iron source to overcome a documented soil nutrient deficiency (Table 4.2).

Citric acid (either synthetic or nonsynthetic) is allowed as a crop production aid when used as a chelating agent, pH adjuster or buffer (Table 4.3).

Calcium and potassium citrate are listed without restrictions (Table 6.3).

Sodium citrate is restricted to use with sausages or milk products (Table 6.3).

Citric acid is also allowed from synthetic or nonsynthetic sources as a component of food grade cleaners, disinfectants and sanitizers without a mandatory removal event (Table 7.3).

CODEX Alimentarius Commission, Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (GL 32-1999)
ftp://ftp.fao.org/docrep/fao/005/Y2772e/Y2772e.pdf
Citric acid is listed in Table 3 as an allowed nonagricultural ingredient for fruit and vegetable products. Sodium citrate is listed in Table 3 for sausages/pasteurization of egg whites/milk products.

Citric acid is listed in Table 4 as a processing aid for pH adjustment.

Calcium and potassium citrate are not listed.

European Economic Community (EEC) Council Regulation, EC No. 834/2007 and 889/2008
http://www.organic-world.net/news-eu-regulation.html
http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/l_189/l_18920070720en00010023.pdf
Citric acid (E330) is allowed as a preservative in animal nutrition products (EC 889/2008 Annex VI).

Citric acid is allowed as an ingredient in cleaning/disinfecting agents used in animal production (EC 889/2008 Annex VII).

Citric acid (E330) is allowed under EC 889/2008 Section A as an ingredient in the preparation of foods of plant origin.

Sodium citrate (E331) is allowed under EC 889/2008 Section A as an ingredient in the preparation of foods of animal origin.

Calcium citrate (E333) is allowed under EC 889/2008 Section A as an ingredient in the preparation of foods of plant origin.

Citric acid is allowed under EC 889/2008 Section B as a processing aid for the regulation of pH in the brine bath in cheese production and for oil production and hydrolysis of starch

Potassium citrate is not listed.

Japan Agricultural Standard (JAS) for Organic Production
http://www.ams.usda.gov/nop/NOP/TradeIssues/JAS.html
Citric acid is allowed, but it is limited to use as a pH adjuster or for processed vegetable products or processed fruit products (Table 1).

Sodium citrate is allowed, but limited to use for dairy products, or for albumen and sausage as low temperature pasteurization (Table 1).

Calcium and potassium citrate are not listed.

International Federation of Organic Agriculture Movements (IFOAM)
http://www.ifoam.org/standard/norms/cover.html
The IFOAM NORMS for Organic Production and Processing allow citric acid as an additive and a processing and post-harvest handling aid in Appendix 4, Table 1. The calcium, potassium and sodium citrates are allowed as additives.

Citric acid is allowed in equipment cleansers and disinfectants (Appendix 4, Table 2).

Citric acid is allowed in Appendix 5 as a substance for pest and disease control and for disinfection of livestock housing and equipment.

Evaluation Questions for Substances to be used in Organic Handling

Evaluation Question #1: Describe the most prevalent processes used to manufacture or formulate the petitioned substance. Further, describe any chemical change that may occur during manufacture or formulation of the petitioned substance when this substance is extracted from naturally occurring plant, animal, or mineral sources (7 U.S.C. § 6502 (21)).

Citric acid was one of the first organic acids identified and isolated. It was first isolated from lemon juice in 1784 by Carl Scheele, a Swedish chemist. It was commercially produced from Italian from about 1826 until 1919, when production shifted to fermentation using *Aspergillus niger*. More recently, the use of *Candida sp*. and the submerged process has increased.

Various chemical syntheses of citric acid have appeared in the chemical and patent literature since the first one based on the reaction of glycerol-derived 1,3-dichloroacetone with cyanide (Grimoux & Adams, 1880). However, none of these has reached a commercial status competitive with fermentation processes (Berovic & Legisa, 2007), as the expense of the precursors has always exceeded the value of the finished product, or the yields have been so low as to be uneconomical.

Many different fermentation processes have been developed over the past century since the discovery that some microbes overproduce citric acid. In 1917 Currie found strains of *A. niger* that, when cultured with low pH values and high levels of sugar and mineral salts, would produce high levels of citric acid instead of the oxalic acid that was previously known as the primary fermentation product. This discovery eventually led to the building of the first domestic production facility in 1923 by Chas. Pfizer & Co. and subsequently more facilities from other companies, all of which used the so-called "surface process" (Milsom 1987; Kristiansen, et al. 1999). Given the widespread use of citric acid, the focus is on developing a cheap process (Kubicek, 2014). Because citric acid is a bulk, low-value product, the market is very competitive, and information about the various commercial processes and procedures is very closely held. Even patents do not provide adequate protection, so much of this production information is cloaked in industrial secrecy (Kristiansen, et al., 1999). About 99% of world production of citric acid occurs via microbial processes, which can be carried out using surface or submerged cultures described in detail below; Max, et al. 2010). The following table describes manufacturing steps using two citric acid production microorganisms.

Table 2. Overview of current citric acid production procedures (Kristiansen, et al. 1999)

Parameter	*Aspergillus niger*		*Candida guilliermondii*
Fermentation type	Surface fermentation (0.05-0.2m)	Submerged fermentation	Submerged fermentation
Fermenter inoculum	Conidia/spores	Spore/seed fermenter	Seed fermenter
Substrate	Molasses or glucose syrup plus additional nutrients and salts		
	150 kg/m³	140-220 kg/m³	up to 280 kg/m³
Substrate pre-treatment	Pre-treatment with HCF or copper ions to reach low manganese concentration		No metal ion pre-treatment required
Fermentation pH	Initially 5.0-7.0 for *A. niger* germination/growth. Drops below 2.0 for citrate production phase		pH 4.5-6.5 for growth. Can fall to ~3.5 for citrate production
Temperature	30˚C		25-37˚C
Aeration	(oxygen transfer, cooling)	0.5-1 vvm	0.5-1 vvm
Other	NH_4^+ stimulates citric acid production		Nitrogen limitation triggers acid

			accumulation
		Mycelial morphology as pellets	Thiamine required for acid accumulation

Microorganisms:
For the past 80+ years, citric acid has been produced on an industrial scale by the fermentation of carbohydrates, initially exclusively by *Aspergillus niger*, but in recent times by *Candida* yeasts as well, with the proportion derived from the *Candida* process increasing. The higher productivity of the yeast-based process suggests it will be the method of choice for any new manufacturing facilities that may be built (Kristiansen, et al. 1999). New information indicates that the bulk of citric acid production currently uses *Aspergillus niger* (Kubicek 2014).

Until early in the last century most citric acid was produced from lemon, although Wehmer described it as a metabolic product of species of *Penicillium* and *Mucor* (1893). Today, most citric acid is produced from fungal fermentation. Species of *Penicillium*, *Aspergillus*, *Mucor*, and *Botrytis*, among others, are known to accumulate citric acid in culture. *A. niger* produces citric acid as a major metabolic end product when grown in a sugar-containing medium at low pH. At higher pH, the organism produces significant amounts of oxalic acid (COOHCOOH). Since the first observations (1917), strains of *A. niger* have dominated others in industrial and experimental use. These organisms are Generally Recognized as Safe (GRAS), are relatively easy to handle, and industry has long experience with their culture (Soccol, et al. 2006). They grow on cheap substrates and give high and consistent yields (Kristiansen, et al. 1999).

Traditional mutant selections of *Aspergillus* and yeast genus *Candida* have almost exclusively been utilized (Berovic & Legisa 2007) for citric acid production, and they remain the choice candidates for the biosynthesis of citric acid (Angumeenal & Venkappayya 2013). They may in fact be the only microorganisms approved by FDA for microbial production of citric acid (21 CFR 184.1033). There are cases where citric acid production might be positively affected by gene manipulation. However, these principles have never been introduced into the process because most of the citric acid is used in the food industry, and companies are concerned about the European ban on genetically engineered food (Kubicek 2014). Even though the final citric acid is the same and does not contain genetically modified DNA, most European food suppliers would not purchase it. Current production is exclusively performed by organisms that are considered "classical" mutants (Kubicek 2014).

The yields are high with these strains anyway, and the unwanted byproducts, gluconic and oxalic acid, can easily be avoided by straightforward classical mutation. In addition, a sexual cycle has now been detected in *A. niger* that could be used for crossing in the future (Kubicek 2014). Potential improvements include speeding up the production rate, removing the sensitivity against manganese ions, and reducing the sensitivity to interruptions in the air supply.

Fermentation methods
Historically, the development of processes for citric acid fermentation can be divided into three phases separated by improvements that increased the yield and the ease of producing citric acid. In the early phase, citric acid production was confined to species of *Penicillium* and *Aspergillus* using stationary or surface culture conditions. The beginning of the second phase consisted of the development of submerged fermentation processes for citric acid production using *Aspergillus sp*. The third stage, which is of recent origin, involves the development of solid-state culture, continuous culture, and multistage fermentation techniques for citric acid production (Angumeenal & Venkappayya 2013).

1. Surface culture method (Milsom 1987; Kristiansen, et al. 1999).
The surface process was the initial industrial process used to produce citric acid via fermentation. Sterile media containing sugar is pumped into stainless steel or aluminum trays arranged in tiers in fermentation chambers where temperature, relative humidity, and circulation of sterile air are controlled. The medium is inoculated with spores of *A. niger* at 28-30° C and 40-60% relative humidity for 8-12 days. Spores germinate and produce a mycelium mat, which grows over the surface of the medium. Monitoring pH and/or total

acid in broth occurs throughout fermentation. At the end of fermentation, the broth is drained and processed for citric acid recovery (described below). Mycelium can be reused for one or two rounds of fermentation. Chambers and trays are sterilized before reuse using water, dilute formaldehyde, and sulfur dioxide.

Solid-state fermentation—also considered a surface process, was first described by Cahn (1935). Citric acid can be produced by fermentation with *A. niger* for 38-60 hours on solid materials containing sucrose or molasses. The resulting good yield (45% of the sugar content of the molasses or 55% of the sucrose in pure sucrose is used) and rapid fermentation are due to the use of a culture medium with a very large surface on which the fungus can develop in contact with the air.

The fermentation medium is infused into cheap, porous solid materials such as sugarcane bagasse, potato, beet, pineapple, or other pulps in an appropriate ratio, and then inoculated with spores. There is not enough carbon in these materials, so additional sugar is typically added. Fermentation occurs at 25-30° C over 6-7 days. Another scheme that has been tried involves immobilizing the mycelium on solid materials such as alginate beads or collagen. Because these processes are labor intensive, they have not seen widespread use. These processes are not typically as efficient as the submerged methods described below. Production rates have been too low to be economically viable.

2a. Submerged culture or deep fermentation process.

These approaches are more commonly used currently. These systems typically consist of four areas: medium preparation; reactor; broth separation and product recovery. The first three will be discussed in a limited sense, because the conditions therein would not affect the acceptability of the citric acid produced, since they are just part of the fermentation process. The numerous inputs into the fermentation broth have been low value agricultural waste products (beet molasses), although some are purer sources (cane/corn sugar) because of the greater ease of purification at the end. The final step, product recovery and purification, will be discussed in depth later on.

All steps of the manufacturing process must be carefully controlled to obtain optimum yield. Medium preparation consists of treatment and sterilization of all inputs. The production of citric acid relative to other side reactions is very sensitive to media conditions, and since the inputs are often not well controlled, the careful adjustment of micronutrients, metals, etc. is crucial to efficient citric acid production. The medium is inoculated in a small batch prior to inoculation in large fermentors. The large fermentors are aerated for 3-5 days at 25-30°C. Often the reactors are held above atmospheric pressure to increase the rate of oxygen transfer into the broth, which increases yield. Spent broth is treated at the end of the fermentation, and mycelial pellets are reused.

This process has advantages of being less labor intensive, giving higher production rates, and using less space.

2b. Two-stage fermentation—also a submerged process

This process involves separate "growth" and "production" stages. Growth medium is inoculated with spores and, after 3-4 days of growth, the mycelium is separated from the solution and added to the fermentation broth. The "production" phase occurs over 3-4 days at 25-30° C with vigorous aeration.

3. The koji process (Soccol, et al. 2006)

This is the solid state equivalent of the surface process discussed above. It is not clear whether this process is unique to Japan and Southeast Asia, where there is a good supply of rice bran and fruit wastes that are part of the starting substrates. The fungal varieties selected for this process have sufficient cellulases and amylases to break down the substrates, although low yields are part of the result. It is done at relatively small scale and with rather low efficiency due to difficulties in controlling the process parameters.

4. Other processes

Although patents for continuous, semi-continuous, and multi-stage processes have been issued, large-scale citric acid production still exclusively uses the surface and submerged processes (Kristiansen, et al. 1999).

Substrate (fermentation medium)

The basic substrate for citric acid fermentation in factories using the surface method of fermentation is beet or cane molasses. Factories using submerged fermentation can, in addition to beet or cane molasses, use a substrate of higher purity such as hydrolyzed starch, technical and pure glucose, refined or raw sugar, or purified and condensed beet or cane juice (Berovic & Legisa 2007). Fermentation substrates include molasses (beet molasses, cane molasses), refined or raw sucrose, syrups, starch, hydrol (paramolasses), alkanes, oils and fats, and cellulose.

Other necessary nutrient ingredients are needed to provide sources of nitrogen, phosphorus and various micro and macro nutrients (Kristiansen, et al. 1999). When high purity carbon sources are used, micronutrient supplements may be necessary for proper growth. Amino acids and ammonium salts and nitrates can be used as nitrogen sources. When molasses (one of the most common inputs) is used, there is adequate nitrogen and micronutrients, and often the levels of micronutrients are actually too high and the main concern is to remove them for optimal growth (Lesniak and Kutermankiewicz, 1990). Sucrose and molasses remain the substrates of choice, with initial sugar levels of 15-18%. Too much sugar leads to excessive residual sugar; too little may lead to lower yields and accumulation of oxalic acid.

Inorganic forms of nitrogen are generally used: $(NH_4)_2SO_4$, NH_4NO_3, other nitrates, or urea. In general, high nitrogen levels prolong vegetative growth and delay the citric acid production phase. Phosphorous levels also have profound effects on the fermentation. As observed for nitrogen, high phosphorous levels promote growth at the expense of citric acid production (Kristiansen, et al. 1999).

Pretreatment of raw materials

Because the concentration of trace metals has such a profound effect on citric acid production, various techniques have been used to reduce trace metals in fermentation media (Kristiansen, et al. 1999). Complete elimination is practically impossible, particularly when raw materials such as molasses are used, but two approaches have had some success: 1) chemical pretreatments to reduce trace metal concentrations, and 2) development of fungal strains able to produce high levels of citric acid in the presence of excess trace metals. Potassium ferrocyanide treatment precipitates iron and zinc and has been extensively used. The chemical is either added directly to the fermentation medium, where too much could be inhibitory to fungal growth, or to the substrate (molasses) prior to inoculation. EDTA has also been used as a chelating agent to reduce the availability of metals (Kristiansen, et al. 1999).

Recovery of citric acid

At the end of fermentation, the medium contains citric acid and various undesirable by-products such as mycelium, other organic acids, mineral salts, proteins, etc. The following steps are necessary for the recovery of citric acid from the fermentation medium.

Depending on the process used, the first step is either the separation of liquid broth from the mycelium, or the precipitation of oxalic acid. Separation of the fermentation broth from fungal mycelia and cells can be done by filtration or centrifugation, or a combination of the two processes. Mycelium may be washed to recover additional citric acid that can constitute up to 15% of the total production (Kristiansen, et al. 1999). Waste mycelia may also be pressed to recover additional broth (Max, et al. 2010).

Oxalic acid is removed by precipitation and then physical removal. Small amounts of lime (CaO) are added to the broth, which, because of the exothermic nature of the reaction with water, heats the broth to 80-90°C. This addition forms $Ca(OH)_2$, which precipitates oxalic acid in the form of insoluble calcium oxalate that is removed as a by-product by filtration or centrifugation. Citric acid remains in solution as the mono-calcium salt (i.e., calcium citrate). If oxalic precipitation is done prior to mycelium separation, this filtration or centrifugation step can also function for the removal of mycelium (Kristiansen, et al. 1999; Max, et al. 2010).

The next step is the purification of citric acid, which can be accomplished by a number of methods. The six most common methods are: precipitation; solvent extraction; adsorption, absorption and ion exchange;

liquid membranes; electrodialysis; and ultrafiltration (Kristiansen, et al. 1999).

Precipitation is the most common purification practice. The principle behind the purification methods involves the precipitation of insoluble tricalcium citrate from the fermentation broth. A number of physical factors determine the efficiency of the precipitation process. These include the citric acid concentration, temperature, pH, and rate of lime addition. The process starts with the previously hot broth after the removal of calcium oxalate. If the concentration of citric acid is below about 15%, then some form of concentration (dewatering) is necessary. Milk of lime containing calcium oxide (180-250 g/L) is gradually added while the temperature is maintained above 90°C and the pH is below but close to 7. Loss of citric acid is minimally 4-5% due to solubility of calcium citrate. Most other impurities remain in solution and may be removed by washing the calcium citrate with minimal amounts of water until no sugars, chlorides or colored materials wash off. The calcium citrate is then filtered off and recovered. This is then treated with sulfuric acid (60-70%) to form citric acid and insoluble calcium sulfate (gypsum). The gypsum is filtered off leaving a solution of 25-30% citric acid. This solution may be filtered with activated carbon to remove impurities and/or purified with ion exchange columns. This purified solution is then evaporated (below 40°C to avoid caramelization), crystallized, centrifuged, and dried to obtain citric acid crystals. If the crystallization occurs below 36.5°C, the monohydrate is formed. Above this temperature it is the anhydrate that may be obtained. A flow chart of the entire process is shown in Figure 3:

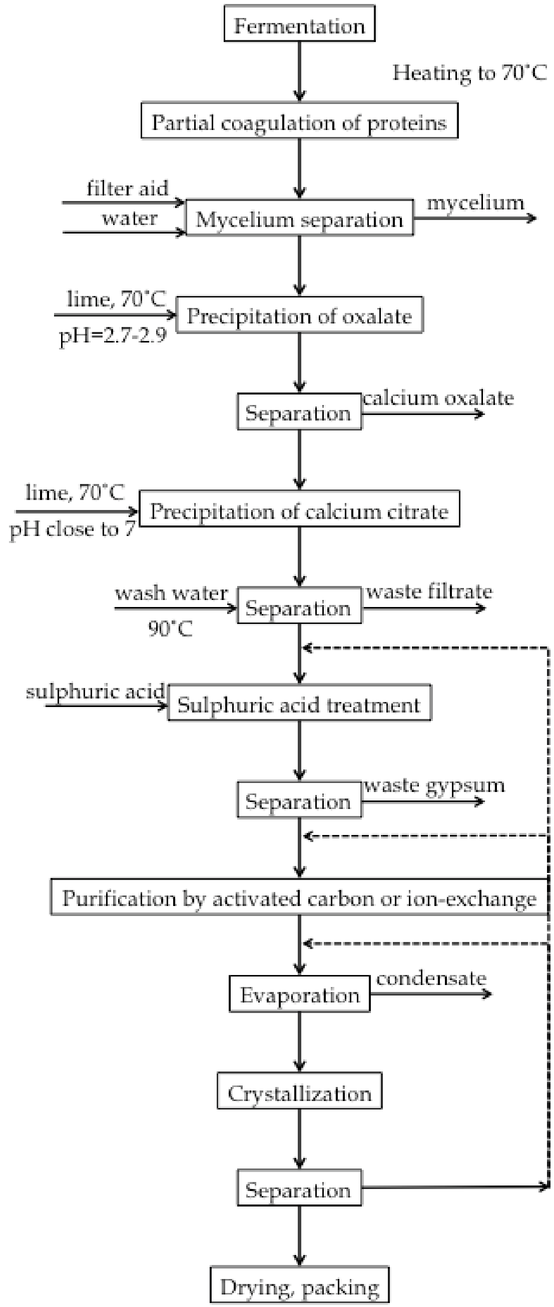


Figure 3. Flow chart of the standard precipitation method of citric acid recovery from fermentation broth (downstream processing in citric acid production; Kristiansen, et al. 1999).

The above process produces a significant amount of waste. For one metric ton of citric acid, 579 kg of calcium hydroxide, 765 kg of sulfuric acid and 18 m3 of water are consumed and approximately one metric ton of gypsum is produced (Berovic & Legisa 2007).

Alternative precipitation processes have been proposed. Ayers (1957) suggested changing the conditions to precipitate di-calcium citrate. This has advantages of reduced chemical usage, lower by-product formation and purer crystals. Schultz (1963) suggested isolating citric acid directly from the fermentation broth by formation of alkali metal salts. Recovery can vary from 50-80% depending on the alkali used. Some use of the standard precipitation process is still required for high yields, but this is performed on much smaller quantities of liquor. Subsequent purification of citric acid may then be performed on cation exchange resins or by electrodialysis.

Lesniak (1989) and Adamczyk, et al. (1985) developed a precipitation method using crystalline sugar as the fermentation source, which, due to its higher purity, allowed direct removal of impurities by coagulating agents and activated carbon followed by filtration. Further purification uses ultrafiltration and ion exchange resins followed by concentration, crystallization and drying like the standard procedure. This process can purify up to 80% of the citric acid present in the original broth, the remainder of which can be recycled back into subsequent batches or processed by the standard method. This method is outlined in the following figure:

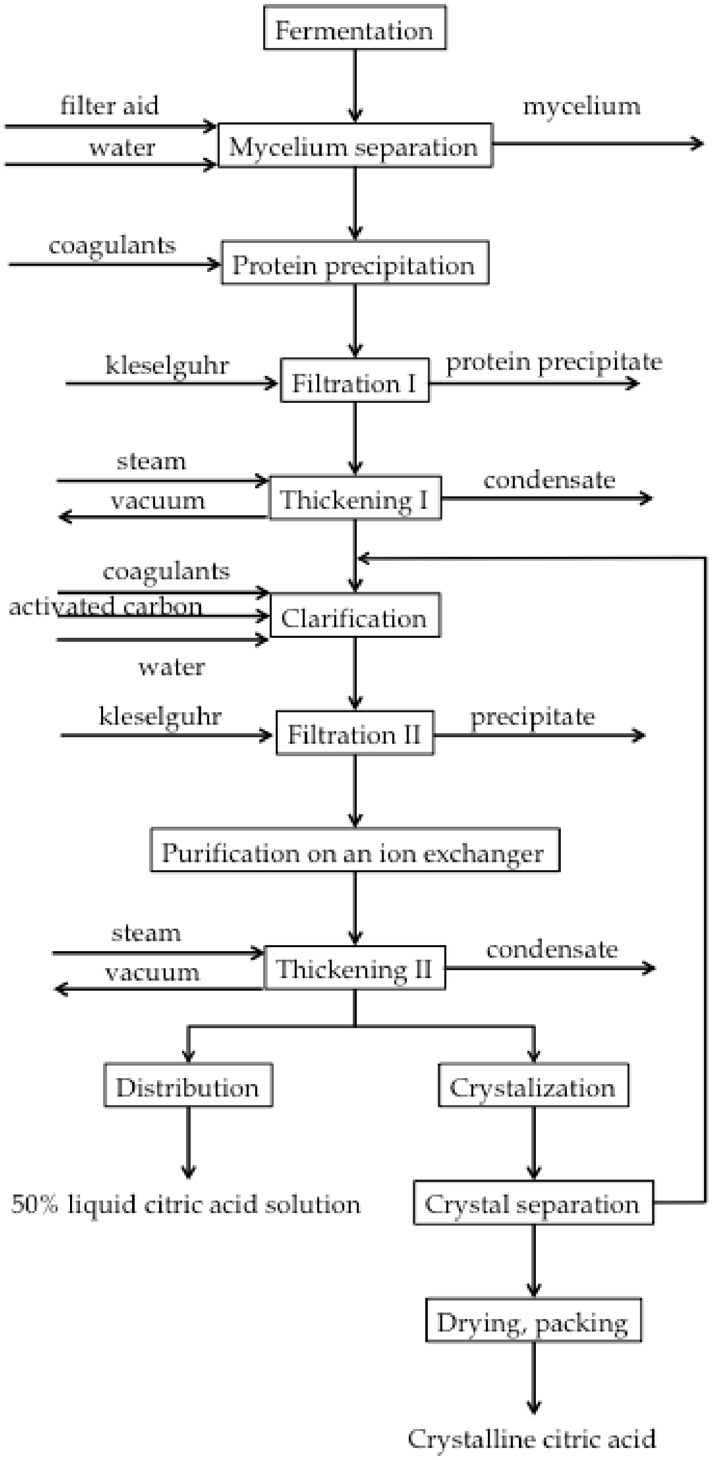


Figure 4. Flow chart of the simplified non-citrate method of citric acid separation and purification (Kristiansen, et al. 1999).

A second method for recovery from the fermentation broth is solvent extraction (Milsom 1987; Hartl & Marr 1993; Kertes & King 1986; Kristiansen, et al. 1999; Schügerl 1994). Extraction schemes use the solubility differences between citric acid and the impurities that one is trying to remove. Three protocols are described:

1) Extraction with organic solvents that are partially or completely immiscible with water (Kasprzycka-Guttman & Kurcińska 1989);

2) Extraction with organophosphorus compounds such as tri-*n*-butylphosphate (TBP) (Pagel & Schwab, 1950) and alkylsulfoxides, e.g., trioctylphosphine oxide (TOPO) (Grinstead 1976; Nikitin, et al. 1974).

3) Extraction with water insoluble amines or a mixture of two or more amines, as a rule dissolved in a substantially water-immiscible organic solvent, and extraction with amine salts (Milsom 1987; Baniel 1982; Bauer, et al. 1988; Bizek, et al. 1992; Juang & Huang 1995; King 1992; Prochazka, et al. 1994).

Concerns regarding solvent extraction of citric acid destined for food use have been raised all along due to teratogenic effects of some of the solvents (Kristiansen, et al. 1999; Kılıç, et al. 2002). Regardless, an amine extraction patented by Baniel, et al. (1981) and Baniel (1982) has received approval by FDA (Milsom 1987; FDA 1975, 21 CFR 173.280, 2014). This is the only method out of many extraction patents that has been applied to large-scale production (Kristiansen, et al. 1999) and it was said to be in use at one plant in the U.S. many years ago (Milsom 1987).

Kılıç, et al. (2002) discussed an extractive fermentation, in which the steps of citric acid production by *A. niger* and separation occur simultaneously, using corn oil and Hostarex A327 in oleic alcohol.

A third means of purification uses adsorption, absorption and ion exchange. Many different schemes have been demonstrated, most of which were not adopted by industry at the time because of difficulties of operation, expense of resin materials and large capital expenses (Kristiansen, et al. 1999). Improvements in this technology could lead to possible adoption, but more recent information from Kubicek, C. (2014) says that this is still not common.

A fourth method involves the use of liquid membranes. These methods have been plagued with a variety of difficulties that prevent their adoption by industry (Kristiansen, et al. 1999). The technology does offer the advantages of lower energy consumption, high separation factors and the ability to concentrate during separation, all in a small physical area. These advantages may lead to eventual adoption of this methodology.

The fifth method is electrodialysis. Pinacci and Radaelli (2002) have proposed the use of bipolar membranes for the recovery of citric acid from fermentation media. This offers an environmentally friendly alternative to the conventional extraction methods. The process enables separation of salts from a solution and their simultaneous conversion into the corresponding acids and bases using electrical potential and mono-or bipolar membranes. The membranes are special ion exchange membranes that, in the presence of an electric field, enable the splitting of water into H^+ and OH^-ions. By integrating bipolar membranes with anionic and cationic exchange membranes, a three- or four-compartment cell may be arranged, in which electrodialytic separation of salt ions, and their conversion into base and acid takes place (Berovic & Legisa 2007). As of 1996 this method had not been applied on an industrial scale, but elimination of environmental problems could lead to adoption of the technology. It also has the potential for the continuous separation of citric acid from fermentation broth (Novalic, et al. 1996). The method is outlined in Figure 5.

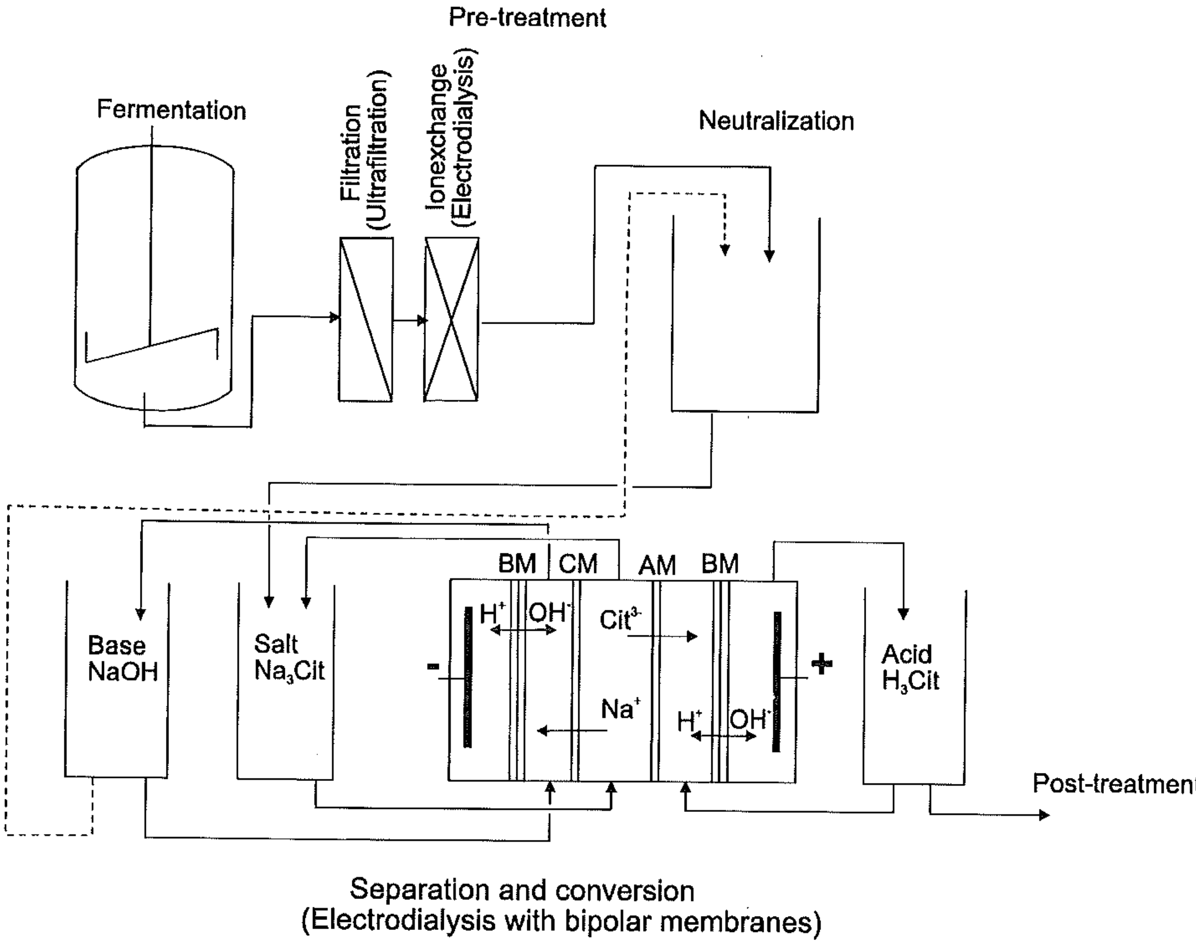


Figure 5. Scheme of citric acid separation by means of electrodialysis with bipolar membranes (Novalic, et al. 1996).

A final method is ultrafiltration and/or nano filtration. Polysulfone membranes with a 10,000 mw cut-off have been used as a first stage, and with a subsequent 200 mw cut-off have yielded promising results (Visacky 1996). This method has the advantages of low energy consumption and no chemical waste in comparison to the standard process, but still requires verification and optimization to be adopted by industry (Kristiansen, et al. 1999).

Given the end use of citric acid, the focus is a cheap process. Therefore, the calcium citrate precipitation method is still used in most cases. The drawback is that the calcium sulfate waste by-product is too contaminated with un-consumed components of the molasses, and with the agents used to antagonize the yield-decreasing metal ions (e.g., hexacyanoferrate, copper), to be used for another purpose.

Citrate Salts

Calcium citrate is the calcium salt of citric acid. It is prepared by neutralizing citric acid with calcium hydroxide or calcium carbonate and subsequent crystallization. It is most commonly found in the tetrahydrate form.

Potassium citrate is the potassium salt of citric acid. It is prepared by neutralizing citric acid with potassium hydroxide or potassium carbonate and subsequent crystallization. It is most commonly found in the monohydrate form.

Sodium citrate is the sodium salt of citric acid. It is prepared by neutralizing citric acid with sodium hydroxide or sodium carbonate and subsequent crystallization. It is most commonly in the anhydrous or dihydrate forms.

Evaluation Question #2: Discuss whether the petitioned substance is formulated or manufactured by a chemical process, or created by naturally occurring biological processes (7 U.S.C. § 6502 (21)). Discuss whether the petitioned substance is derived from an agricultural source.

Naturally occurring biological processes

The industrial production of citric acid is dominated by fermentation by *A. niger* or *Candida spp.* that have been selected for their over-production of citric acid. There has been some historical production of citric acid from lemon juice, but whether this is still being done on an industrial or commercial scale is unknown (Kubicek 2014). There have been some attempts to recover citric acid from pineapple canning waste, but they have not proven to be economical (Ward 1989).

Citric acid is overproduced due to faulty operation of the tricarboxylic acid cycle (TCA, also known as the citric acid cycle or Kreb's cycle) in a variety of organisms Kristiansent, et al. 1999). TCA is a cycle involving the terminal steps in the conversion of carbohydrates, proteins and fats to carbon dioxide and water with concomitant release of energy for growth, movement, luminescence, etc. Studies on the enzyme content of *A. niger* in relation to citric acid accumulation have pointed to the vital role played by the TCA cycle in fermentation (Kristiansent, et al. 1999).

Citric acid production and excretion are independent processes (Kristiansen, et al. 1999). Biological formation of citric acid is purely enzymatic. Under suitable environmental conditions, different species of *Candida* can also produce citric acid (Angumeenal & Venkappayya 2013).

The *Aspergillus* and *Candida* species that are being used for citric acid production have been selected from wild variants with the above mentioned mutations in their TCA cycle metabolism, such that they produce economically useful excess amounts of citric acid.

An agricultural source

A nonagricultural substance is defined under §205.2 as: "A substance that is not a product of agriculture, such as a mineral or a bacterial culture that is used as an ingredient in an agricultural product. For the purposes of this part, a nonagricultural ingredient also includes any substance, such as gums, citric acid, or pectin, that is extracted from, isolated from, or a fraction of an agricultural product so that the identity of the agricultural product is unrecognizable in the extract, isolate, or fraction" (USDA 2014).

Citric acid is cited in the regulations as an example of a nonagricultural substance. It is produced by the fermentation of agricultural materials (see below) which is a naturally occurring biological process as described in the Draft Classification of Materials (NOP 5033-1; NOP 2013).

Molasses, long considered a waste product of the sugar industry, is now termed as a by-product due to its low price compared to other sugar sources, and the presence of minerals and organic and inorganic compounds. Molasses is used in the production of alcohol, organic acid and single cell proteins (Angumeenal & Venkappayya 2013).

The organic and inorganic components present in molasses may inhibit the fermentation process, and hence this material needs to be treated to make it suitable for citric acid production. Some of the commonly followed procedures include treatment with ferrocyanide (El-Naby & Saad 1996), sulfuric acid, tricalcium phosphate, tricalcium phosphate with HCl, and tricalcium phosphate with HCl followed by Sephadex fractionation (Kundu, et al. 1984). Molasses is a more efficient substrate when treated with ammonium oxalate, followed by treatment with diammonium phosphate. Molasses treated by this method was found to serve as a better substrate in producing citric acid, compared to other methods commonly practiced (Angumeenal & Venkappayya 2005c). The above molasses medium and supplementation with selective metal ions as stimulants made citric acid fermentation more successful.

Agro-industrial wastes are frequently used as inexpensive sources of substrates for fermentation. Apple pomace (Hang & Woodams 1984), carob pod (Roukas 1998), carrot waste (Garg & Hang 1995), coffee husk

(Shankaranand & Lonsane 1994), corn cobs (Hang & Woodams 1998), grape pomace (Hang & Woodams 1985), kiwi fruit peel (Hang, et al. 1987), kumara (Lu, et al. 1997), orange waste (Aravantinos, Zafiris, et al. 1994), date syrup (Moataza 2006; Roukas & Kotzekidou 1997), pineapple waste (Tran, et al. 1998), banana extract (Sassi, et al. 1991), potato chips waste, and pumpkin were tried successfully as substrates for citric acid formation. Pumpkin, either as a single or a mixed substrate with molasses, is known to produce good quantities of citric acid (Majumder, et al. 2010).

A waste from jackfruit was also found to be a good and economical substrate for citric acid fermentation. *Artocarpus heterophyllus* (Jackfruit) is a large tree grown in tropical countries and is one of the common fruits in South India. The fruiting perianths (bulbs), seeds and rind constitute 29%, 12% and 59% of the ripe fruit, respectively. The rind portion includes the carpel fiber that holds the fruity portion intact. Chemical analysis of this carpel fiber indicates the presence of sugars and minerals, and hence the fiber was used as a substrate for citric acid production. Batch fermentation using *A. niger* was followed and the results indicate that jackfruit carpel fiber can serve as a substrate for citric acid production (Angumeenal & Venkappayya 2005a, 2005b, 2005c). When this substrate is completely analyzed and the limiting substances identified, steps can be taken to remove them and make it a more efficient substrate for citric acid fermentation.

Tuber crops belonging to the family Araceae, namely *Colocassia antiquorum*, *Aponogetannatans* and *Amorphophallus campanulatus,* are cultivated in large quantities for their edible portion. These tubers were chemically treated and utilized as substrates for citrate production by fermentation using *A. niger* in batch fermentation, and their fermentation capabilities were improved in research trials by adding trace elements (cadmium, molybdenum, chromium and lead) in optimum quantities (Angumeenal, et al. 2003a, 2003b). When *A. campanulatus* was used as a substrate, succinic acid was also produced in high amounts. In fact, the amount of succinic acid produced was higher than the citric acid. This was due to the increased activity of aconitase in the later stages of fermentation. Hence, this substrate can be further explored for succinic acid production using some growth promoters. The potential of *A. campanulatus* in producing citric acid was enhanced by the addition of metal ions.

The citrate salts, although based on agriculturally-derived citric acid, have gone through a synthetic process and are thus considered synthetic, nonagricultural materials.

<u>Evaluation Question #3:</u> If the substance is a synthetic substance, provide a list of nonsynthetic or natural source(s) of the petitioned substance (7 CFR § 205.600 (b) (1)).

Citric acid is listed as a nonsynthetic at §205.605(a) of the National List. Although it has been isolated from citrus fruits, the primary manufacturing process is by fermentation which is considered nonsynthetic.

The citrate salts are listed as synthetic at §205.605(b) of the National List. Although many citrus and acid fruits contain naturally occurring citrate salts, the literature does not suggest that the salts are extracted from fruit. Rather, the commercial method of producing pure forms of citrates is via synthetic chemical reaction of citric acid with the respective alkali substances (e.g., sodium, calcium or potassium hydroxide).

<u>Evaluation Question #4:</u> Specify whether the petitioned substance is categorized as generally recognized as safe (GRAS) when used according to FDA's good manufacturing practices (7 CFR § 205.600 (b)(5)). If not categorized as GRAS, describe the regulatory status.

Citric acid and the citrate salts are all generally recognized as safe (GRAS).

Citric acid is listed as GRAS in CFR Title 21 Part 184.1033. Calcium citrate is GRAS as listed at §184.1195. Potassium citrate is GRAS as listed at §184.1625. Sodium citrate is GRAS as listed at §184.1751.

<u>Evaluation Question #5:</u> Describe whether the primary technical function or purpose of the petitioned substance is a preservative. If so, provide a detailed description of its mechanism as a preservative (7 CFR § 205.600 (b)(4)).

A chemical food preservative is defined under FDA regulations at 21 CFR 101.22(a)(5) as "any chemical that, when added to food, tends to prevent or retard deterioration thereof, but does not include common salt, sugars, vinegars, spices, or oils extracted from spices, substances added to food by direct exposure thereof to wood smoke, or chemicals applied for their insecticidal or herbicidal properties." Citric acid has a wide variety of uses, some of which can provide preservative functions, primarily through lowering the pH of the food. Proper pH control has been known for a very long time as a food safety measure, and citric acid has played a significant role in adjusting pH to prevent the growth of organisms such as *C. botulinum*. It is the lowering of the pH (by citric acid), not the citric acid itself, that provides this important food safety function.

The citrate salts have similar pH lowering effects, although to a much lesser degree. They are not often used for this function.

The most common classical preservative agents are the weak organic acids, for example acetic, lactic, benzoic and sorbic acid (Brul & Coote 1999). These molecules inhibit the outgrowth of both bacterial and fungal cells, and sorbic acid is also reported to inhibit the germination and outgrowth of bacterial spores (Blocher & Busta 1985; Sofos & Busta 1981).

Citrate (not specified as free acid or salt) is very effective against the gram-positive bacteria *L. monocytogenes* and *Listeria innocua* (Jones, et al. 1990; Ter Steeg 1993).

Chelators that can be used as food additives include the naturally occurring acid, citric acid, and the disodium and calcium salts of ethylenediaminetetraaccetic acid (EDTA) (Russell 1991). EDTA is known to potentiate the effect of weak acid preservatives against gram-negative bacteria, while citric acid inhibits growth of proteolytic *C. botulinum* due to its CA^{2+} chelating activity (Graham & Lund 1986). Helander, et al. (1997) discussed the role of chelators as permeabilising agents of the outer membrane of gram-negative bacteria. Indeed, exposure to citric acid is well known to potentiate the effect of glycerol monolaurate (an emulsifier) against gram-negative bacteria (Shibasaki & Kato 2010).

Blaszyk and Holley (1998) state "The presence of sodium citrate was necessary to yield potent inhibition of *Lactobacillus curvatus* and *Lb. sake* growth by the monolaurin and eugenol combinations."

About 70% of the citric acid market is in the food and beverage industry. Major attractions of citric acid as a food and beverage acidulant are high solubility, extremely low toxicity, and pleasant sour taste (Karaffa & Kubicek 2003; Kristiansen, et al. 1999). Berovic & Legisa (2007) also state that citric acid is used mainly in the food and beverage industry, primarily as an acidulant.

Citric acid is mainly used in the food and beverage industry, because of its general recognition as safe, and having pleasant taste, high water solubility, and chelating and buffering properties. Citric acid is used extensively in carbonated beverages to provide taste and to complement fruit and berry flavors. It also increases the effectiveness of antimicrobial preservatives. The amount of acid used depends on the flavor of the product. It usually varies from 1.5 to 5%.

In jams and jellies it is used for taste and for pH adjustment in the final product. For optimum gelation, pH has to be adjusted within very narrow limits. Citric acid is usually added as a 50% solution to assure good distribution through the batch. The chelating and pH adjusting properties of citric acid enable it to optimize the stability of frozen food products by enhancing the action of antioxidants, and by inactivating enzymes. It also helps to prolong the shelf life of frozen fish and shellfish.

Citric acid also inhibits color and flavor deterioration in frozen fruit. Amounts in concentration of 0.005–0.02% citric acid are used as an antioxidant synergism in fats, oils and fat-containing foods.

Citric acid is the principal food acid, used in the preparation of soft drinks and syrups, desserts, jams, jellies, wines, candy, preserved fruits, frozen fruits and vegetable juices. Citric acid is also used in gelatin food products and artificial flavors of dry compounds for materials such as soft drink tablets and powders (Ashy & Abou-Zeid 1982).

The product is sold as an anhydrous or monohydrate acid, and about 70% of total production of 1.5 million tons per year is used in the food and beverage industry as an acidifier or antioxidant to preserve or enhance the flavors and aromas of fruit juices, ice cream and marmalades. About 20% is used, as such, in the pharmaceutical industry as an antioxidant to preserve vitamins, effervescence, as a pH corrector or blood preservative, or in the form of iron citrate as a source of iron for the body, as well as in tablets, ointments and cosmetic preparations (Max, et al. 2010).

<u>Evaluation Question #6:</u> Describe whether the petitioned substance will be used primarily to recreate or improve flavors, colors, textures, or nutritive values lost in processing (except when required by law) and how the substance recreates or improves any of these food/feed characteristics (7 CFR § 205.600 (b)(4)).

Due to its versatile array of food uses, it is difficult to determine whether citric acid and its salts are used primarily to recreate flavors and textures lost in processing, although it is clear that they are used indirectly for these purposes. For example, citric acid is used extensively in carbonated beverages to provide a sour taste and to complement fruit and berry flavors. It also increases the effectiveness of antimicrobial preservatives. The amount of acid used depends on the flavor of the product. It usually varies from 1.5- 5% (Berovic & Legisa 2007). In jams and jellies it is used for taste and for pH adjustment in the final product. For optimum gelation, pH has to be adjusted within very narrow limits (Crueger & Crueger 1984). Citric acid is usually added as a 50% solution to assure good distribution through the batch. In the confectionery industry 0.5–2% is used as a flowing agent. The chelating and pH adjusting properties of citric acid enable it to optimize the stability of frozen food products by enhancing the action of antioxidants, and by inactivating enzymes. It also helps to prolong the shelf life of frozen fish and shellfish. These are all examples of how citric acid indirectly affects flavors, textures, and nutritive values in foods, although these characteristics may not have been lost due to processing.

In addition, the use of 10 mmol $litre^{-1}$ glutathione and 100 mmol $litre^{-1}$ citric acid was found to give good control of the browning of litchi fruit and 80–85% inhibition of PPO observed. Application of glutathione in combination with citric acid is recommended as a way of slowing the browning of litchi fruit (Jiang & Fu 1998).

Citric acid also inhibits color and flavor deterioration in frozen fruit. Amounts in concentration of 0.005–0.02% citric acid are used as an antioxidant synergism in fats, oils and fat-containing foods. As a flavor adjunct, citric acid is used in sherbets and ice creams.

Potassium citrate is commonly used in biscuits, baby food, soup mixes, soft drinks, and fermented meat products. Sodium citrate is chiefly used as a food additive, usually for flavoring or as a preservative. Sodium citrate gives club soda both its sour and salty flavors. It is common in lemon-lime soft drinks, and it is partly what causes them to have their sour taste. Additionally, it is used in jams, jellies, meat products, baby foods, and milk powder.

Calcium citrate may be added to foods to supplement calcium per FDA nutrition guidelines, although there are other calcium sources for supplementation purposes including calcium carbonate, calcium oxide, calcium sulfate, etc., all of which are permitted per a separate listing on 205.605(b) as Nutrient Vitamins and Minerals.

<u>Evaluation Question #7</u>: Describe any effect or potential effect on the nutritional quality of the food or feed when the petitioned substance is used (7 CFR § 205.600 (b)(3)).

In recent years, a number of studies have reported on attempts to improve bioavailability of calcium by the addition of compounds such as citric acid (Bronner & Pansu 1999; Lacour, et al. 1996).

Iron bioavailability is normally somewhat impaired when simultaneously administered with calcium, but this impairment is overcome when organic acids (citric and malic) and vitamin C are included in the vitamin and mineral supplemented beverages (Heckert, et al. 1991).

Evaluation Question #8: List any reported residues of heavy metals or other contaminants in excess of FDA tolerances that are present or have been reported in the petitioned substance (7 CFR § 205.600 (b)(5)).

Metals from the incoming agricultural feedstocks have been a problem with efficient fermentations, so they are often reduced by preprocessing of these feedstocks to reduce metal content (Kristiansen, et al. 1999).The finished products would be subject to good manufacturing practice requirements. No other requirements could be found, but heavy metal content would be expected to be low because of issues with metal content interfering with citric acid production by the fermentation organisms. Refer to Table 2 for treatment of fermentation substrate to reduce metal content of incoming materials.

Evaluation Question #9: Discuss and summarize findings on whether the manufacture and use of the petitioned substance may be harmful to the environment or biodiversity (7 U.S.C. § 6517 (c) (1) (A) (i) and 7 U.S.C. § 6517 (c) (2) (A) (i)).

The fermentation process is advantageous as it is based on renewable sources, it facilitates use of waste for productive purpose, and useful by-products are created. It involves very mild, environmentally-friendly conditions described below, and also consumes less energy than other production methods. It also faces some drawbacks including:

1) Uses of large quantities of water. For one metric ton (2200 lbs.) of citric acid, approximately 18m^3 (4000 gal.) of water are required (Kristiansen, et al. 1999).

2) Due to high BOD (Biochemical oxygen demand) the waste requires treatment before disposal (Angumeenal & Venkappayya 2013).

3) The citric acid purification process produces significant waste. For one metric ton of citric acid, 579 kg of calcium hydroxide, 765 kg of sulfuric acid and 18 m^3 of water are consumed, and approximately one metric ton of gypsum are produced (Berovic & Legisa 2007).

4) Waste calcium sulfate from the purification process is too dirty (it contains most of the non-consumed components of the molasses including herbicides, etc.) and contaminated (with the agents used to antagonize the yield-decreasing metal ions, such as hexacyanoferrate, copper, etc.) to be used for any purpose, and thus has to be deposited in the (mostly nearby) soil, creating an environmental hazard (Kubicek 2014).

On the other hand, the citric acid production also exhibits some characteristics of an environmentally friendly chemical, such as:

1) Citric acid, trisodium salt is readily biodegradable. In a ready biodegradation test, using sewage from a waste water treatment plant as the inoculum, sodium citrate degraded 90% in 30 days. (EPA 2007).

2) The log K_{ow} values of citric acid and citrate salts indicate that the potential to bioaccumulate is low. Citric acid and citrate salts are readily biodegradable, indicating that they are not expected to persist in the environment (EPA 2007).

3)It was possible to control simultaneous production of pectinolytic, cellulolytic and xylanolytic enzymes by fungal strains of the genera *Aspergillus*, *Fusarium*, *Neurospora* and *Penicillium*. The process generated multi-enzyme activities using a simple growth medium consisting of a solid by-product of the citrus processing industry (orange peels) and a mineral medium. Furthermore, the two-stage process proposed, which includes coupling enzymatic treatment and solid-state fermentation, resulted in the production of fermentable sugars that could be converted to bioethanol (Mamma, et al. 2008).

Evaluation Question #10: Describe and summarize any reported effects upon human health from use of the petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (i), 7 U.S.C. § 6517 (c) (2) (A) (i)) and 7 U.S.C. § 6518 (m) (4)).

Based on various toxicology studies, citric acid and its salts are not expected to pose any significant health hazard upon ingestion, although citric acid is considered a severe eye irritant and moderate skin irritant in its pure state (EPA 1992). Following is a sample of various toxicology studies conducted with citric acid and its salts:

The acute oral toxicity for citric acid and its salts is low. Dermal acute exposure of citric acid caused erythema and edema in rabbits at 50 mg/kg-bw. Repeated exposures to this subcategory via the oral route showed no gross or histopathological changes or effects on growth or survival at 5% (approximately 1500 mg/kg-bw/day) in New Zealand albino rabbits. In a 6-week dosed feed experiment, a no-observed-adverse-effect level (NOAEL) of 2260 mg/kg bw/day and a lowest-observed-adverse-effect level (LOAEL) of 4670 mg/kg-bw/day were determined for rats. Citric acid and its salts were not mutagenic in tested strains of *S. typhimurium*. No data are available for chromosomal aberration (EPA 2007).

The potential health hazard of citric acid and citrate salts category is moderate based on systemic toxicity (EPA 2007). EPA listed citric acid and the salts as List 4A (minimal risk inert) in their 2004 list.

Citric acid

In a 6-week repeated-dose toxicity study, 10 Sprague-Dawley male rats/concentration were fed diet containing 0, 0.2, 2.4 and 4.8% (approximately 200, 2400 and 4800 mg/kg-bw/day) citric acid. No behavioral abnormalities, effects on body weight gain or mortality were observed. Some minor biochemical changes were observed at the highest dose, but no specific histopathological abnormalities were detected.
LOAEL = 4670 mg/kg-bw/day (based on some minor biochemical changes observed at the highest dose)
NOAEL = 2260 mg/kg-bw/day

Sodium citrate:

(1) In a 1-year oral repeated-dose toxicity study, two successive generations of rats were exposed to 0.1% citric acid, sodium salt (approximately 50 mg/kg-bw/day) in the diet. No adverse effects were seen in rats. A limited number of tissues were examined microscopically.
LOAEL > 0.1% citric acid, sodium salt (approximately 50 mg/kg-bw/day based on no effects at one concentration)
NOAEL = 0.1% citric acid, sodium salt

(2) In a 32-week oral repeated-dose toxicity study, 20 male rats (species not stated) were treated with 5% citric acid, sodium salt (about 2,500 mg/kg-bw/day) in the diet. No overt signs of toxicity were observed.
LOAEL > 2500 mg/kg-bw/day (based on no effects at the only concentration tested)
NOAEL = 2500 mg/kg-bw/day

<u>Reproductive Toxicity</u>

Citric acid:

(1) In a fertility study, rats (species, number of animals not stated) were exposed to 1.2% citric acid (approximately 600 mg/kg-bw/day) in their daily diet. No data on control group use is available for this study. Exposure began 29 weeks prior to mating and continued for a few months after mating. There were no detectable reproductive toxic effects (only limited information is available).
LOAEL for systemic toxicity > 600 mg/kg-bw/day (based on no observed effects)
NOAEL for systemic toxicity = 600 mg/kg-bw/day
LOAEL for reproductive toxicity > 600 mg/kg-bw/day (based on no treatment-related effects)
NOAEL for reproductive toxicity = 600 mg/kg-bw/day

(2) In a one-generation oral reproductive toxicity study, rats (species not stated) (24/sex/dose) and mice (24/sex/dose) were treated with 5% citric acid (about 2500 mg/kg-bw/day) citric acid in their daily diet. Body weight gain and mean survival was markedly reduced when compared to the control groups. Effects on body weight gain and survival time may have resulted from the chelating ability of citric acid, which could reduce the physiological availability (absorption) of calcium and iron present at dietary marginal levels. No effects were seen on number of pregnancies, number of young born, or survival of young in either mice or rats.
LOAEL for systemic toxicity = 2500 mg/kg-bw/day (based on decreased body weight gain and mean survival times of male mice)
NOAEL for systemic toxicity = Not established
LOAEL for reproductive toxicity > 2500 mg/kg-bw/day (based on no treatment-related effects on reproduction)
NOAEL for reproductive toxicity = 2500 mg/kg-bw/day

Sodium citrate
In a fertility study, rats (species, number of animals not stated) were exposed to 0.1% citric acid, sodium salt (approximately 50 mg/kg-bw/day) in their daily diet. Exposure began 29 weeks prior to mating and continued for a few months after mating. No reproductive effects were detected.
LOAEL for systemic toxicity > 0.1% (approximately 50 mg/kg-bw/day, based on no treatment-related effects)
NOAEL for systemic toxicity = 0.1% (approximately 50 mg/kg-bw/day)
LOAEL for reproductive toxicity > 0.1% (approximately 50 mg/kg-bw/day, based on no treatment-related effects on reproduction)
NOAEL for reproductive toxicity = 0.1% (approximately 50 mg/kg-bw/day)

Developmental Toxicity

Citric acid
In a developmental toxicity study, pregnant rats (species and number of animals not stated) were exposed to 241 mg/kg-bw/day citric acid by oral gavage daily on days 6 – 15 of gestation. No information was provided on control group. No adverse effects were observed on fertilization, maternal, or fetal survival.
LOAEL for maternal and developmental toxicity > 241 mg/kg-bw/day (based on no observed effects at the only dose level tested)
NOAEL for maternal and developmental toxicity = 241 mg/kg-bw/day (based on no observed effects at the only dose level tested).

Based on many experimental data in animals and on human experience, citric acid is of low acute toxicity. The NOAEL for repeated dose toxicity for rats is 1200 mg/kg/d. The major, reversible (sub) chronic toxic effects seem to be limited to changes in blood chemistry and metal absorption/excretion kinetics. Citric acid is not suspected of being a carcinogen nor a reprotoxic or teratogenic agent. The NOAEL for reproductive toxicity for rats is 2500 mg/kg/d. (UNEP 2001).

In several *in vitro* and *in vivo* tests, citric acid was not mutagenic (Türkoğlu, Ş. 2007).

Citric acid and its salts may also have beneficial health affects in humans. For example beverages containing citric acid may be useful in nutrition therapy for calcium urolithiasis (urinary or kidney stones), especially among patients with hypocitraturia. Citrate is an inhibitor of urinary crystallization; achieving therapeutic urinary citrate concentration is one clinical target in the medical management of calcium urolithiasis. When provided as fluids, beverages containing citric acid add to the total volume of urine, reducing its saturation of calcium and other crystals, and may enhance urinary citrate excretion. Citrate salts of various metals are used to deliver minerals in biologically available forms; examples include dietary supplements and medications (Penniston, et al. 2008).

Urinary citrate is a potent, naturally occurring inhibitor of urinary crystallization. Citrate is freely filtered in the proximal tubule of the kidney. Approximately 10- 35% of urinary citrate is excreted; the remainder is absorbed in various ways, depending on urine pH and other intra-renal factors. Citrate is the most abundant organic ion found in urine. Hypocitraturia, defined as <320 mg (1.67 mmol) urinary citrate/day, is a major risk factor for calcium urolithiasis. The activity of citrate is thought to be related to its concentration in urine, where it exhibits a dual action, opposing crystal formation by both thermodynamic and kinetic mechanisms. Citrate retards stone formation by inhibiting the calcium oxalate nucleation process and the growth of both calcium oxalate and calcium phosphate stones, largely by its ability to bind with urinary calcium and reduce the free calcium concentration, thereby reducing the supersaturation of urine. Citrate binds to the calcium oxalate crystal surface, inhibiting crystal growth and aggregation. There is also evidence that citrate blocks the adhesion of calcium oxalate monohydrate crystals to renal epithelial cells. Medical interventions to increase urinary citrate are a primary focus in the medical management of urolithiasis.

The amount of diet-derived citrate that may escape *in vivo* conversion to bicarbonate is reportedly minor (Meschi, et al. 2004). Nonetheless, a prior study (Seltzer et al. 1996) reported increased urinary citrate after 1 week on 4 ounces of lemon juice per day, diluted in 2 L water, in stone formers with hypocitraturia. Two retrospective studies showed an effect in calcium stone formers of lemon juice and/or lemonade consumption on urinary citrate, but a recent clinical trial showed no influence of lemonade on urinary citrate (Penniston, et al. 2008).

Koff, et al. (2007) found that potassium citrate improves citrate levels and urinary pH to a significant degree, but patients had a significantly decreased urine volume compared with their urine volume drinking lemonade. Uric acid levels in urine were not affected by consuming lemonade or potassium citrate

<u>Evaluation Question #11:</u> **Describe any alternative practices that would make the use of the petitioned substance unnecessary (7 U.S.C. § 6518 (m) (6)).**

Due to the versatility of citric acid and its salts, there are no practices that could be used to substitute for all functions they provide. Rather, there are some possible alternative substances that can be used in instead, and these are described in Question #12 and #13 below.

<u>Evaluation Question #12:</u> **Describe all natural (non-synthetic) substances or products which may be used in place of a petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (ii)). Provide a list of allowed substances that may be used in place of the petitioned substance (7 U.S.C. § 6518 (m) (6)).**

There has been some historical production of citric acid from lemon juice, but this is no longer being done on an industrial or commercial scale (Kubicek 2014). There have been some attempts in the past to purify citric acid from pineapple canning waste, but this has not proven economically competitive with fermentation sources (Ward 1989).

Citric acid purified from citrus fruits is technically feasible, but whether it is economically possible is unknown. Since the fermentation process used for the current manufacture of citric acid is considered a natural source, the question of production from citrus may be a moot point, although depending on the purification process used (electrodialysis or ultra/nano filtration), it may be possible to get a certified organic citric acid from a certified organic citrus source.

Among fruits, citric acid is most concentrated in lemons and limes, comprising as much as 8% of the dry fruit weight. Lemon and lime juice are rich sources of citric acid, containing 1.44 and 1.38 g/oz., respectively. Lemon and lime juice concentrates contain 1.10 and 1.06 g/oz., respectively. The citric acid content of commercially available lemonade and other juice products varies widely, ranging from 0.03 to 0.22 g/oz. (Penniston, et al., 2008). These juice products are possible alternatives, but are not widely used because of the flavor impact associated with them.

For supplying calcium as a nutritive supplement, natural, mined calcium sulfate and calcium carbonate can be used in place of calcium citrate, as well as calcium chloride derived from brines. These substances appear on §205.605(a) as nonsynthetic substances allowed for use in organic products.

Otherwise, there are no nonsynthetic sources or alternatives for the other uses of the citrate salts.

<u>Evaluation Information #13:</u> Provide a list of organic agricultural products that could be alternatives for the petitioned substance (7 CFR § 205.600 (b) (1)).

There are currently no organic agricultural products that could be used in place of citric acid. The citrate salts are synthetic and have no agricultural organic alternatives.

There has been some historical production of citric acid from lemon juice, but this is apparently no longer being done on an industrial or commercial scale (Kubicek, 2014). There have been some attempts in the past to purify citric acid from pineapple canning waste, but this has not proven economically competitive with fermentation sources (Ward, 1989).

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There are no nonsynthetic sources or alternatives for the citrate salts.

Citrus fruits, juices, and wine may be added directly to recipes in place of purified citric acid, as they contain high concentrations of citric acid. These citrus sources are not always suitable substitutes for purified or crystallized forms. Table 3 shows the different sugar and acid contents of orange juice and wine.

Table 3. Sugar and organic acid compositions of orange juice and wine (Kelebek, et al. 2009).

Compound	Orange juice	Wine
Sugars (g/L)		
Sucrose	59.34±2.04	44.68±1.27
Glucose	32.30±0.86	1.06±0.36
Fructose	28.55±0.94	3.04±1.08
Total	120.19±3.84	48.78±2.71
Non-volatile Organic acids (g/L)		
Citric acid	12.66±0.16	6.03±0.08
Ascorbic acid	0.49±0.01	0.23±0.01
Malic acid	1.06±0.01	0.34±0.01
Total	14.21±0.18	6.60±1.01

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